nant magnetic carrier in the sheeted dike basalts, did not form simply by oxidation-exsolution, as has commonly been assumed (*6*, *12*, *26*). The natural remanent magnetization (NRM) of the sheeted-dike basalts was presumably acquired by the single-domain Ti-bearing magnetite (the host after exsolution) during initial cooling. This was a thermoremanent magnetization (TRM) and was then modified by chemical remanent magnetization of recrystallized end-member magnetite during hydrothermal alteration near the spreading center. The initial cooling and hydrothermal alteration appear to have taken place soon after the intrusion of basalts; therefore, the NRM reflects the original geomagnetic field direction (*6*).

The thickness of the source layers responsible for the sea-floor magnetic anomalies has long been debated and has been estimated as extending from the uppermost 500 to 1000 m (pillow basalts, layer 2A) of the oceanic crust to depths of ~8 km (essentially the entire oceanic crust) (*3*, *7*, *27*, *28*). The results of studies of magnetic properties of sheeted dike basalts recovered from DSDP drill holes suggest that the sheeted dike complex (layer 2B) contributes significantly to sea-floor magnetic anomalies (*6*, *26*, *29*). However, magnetic data from ocean gabbros indicate that the linear magnetic anomalies originated partly in the gabbro layer (layer 3) (*29*–*31*). We have shown that single-domain, end-member magnetite, an efficient and stable carrier of TRM, is responsible for the magnetic properties in the upper levels (depths of 630 m to at least −1125 m within the igneous basement) of the sheeted dike complex at site 504B. The resultant NRM intensity of the sheeted dike basalts is on the same order as that of the pillow basalts at site 504B (*6*, *8*, *12*). We therefore conclude that the upper sheeted dike basalts from DSDP hole 504B are a significant source of sea-floor magnetic anomalies.

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Induction of Apoptosis by the Low-Affinity NGF Receptor

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Nerve growth factor (NGF) binding to cellular receptors is required for the survival of some neural cells. In contrast to Trk A, the high-affinity NGF receptor that transduces NGF signals for survival and differentiation, the function of the low-affinity NGF receptor, $p75^{NGFR}$, remains uncertain. Expression of $p75^{NGFR}$ induced neural cell death constitutively when $p75^{NGFR}$ was unbound; binding by NGF or monoclonal antibody, however, inhibited cell death induced by $p75^{NGFR}$. Thus, expression of $p75^{NGFR}$ may explain the dependence of some neural cells on NGF for survival. These findings also suggest that $p75^{NGFR}$ has some functional similarities to other members of a superfamily of receptors that include tumor necrosis factor receptors, Fas (Apo-1), and CD40.

Growth factors such as NGF enhance the survival of cells displaying the appropriate receptors. The effects of NGF are mediated at least in part by Trk A, the high-affinity NGF receptor, which is a tyrosine kinase (*1*). The low-affinity NGF receptor, $p75^{NGFR}$, is a receptor of incompletely characterized function: $p75^{NGFR}$ has been shown to increase the affinity of Trk A for NGF (*1*) and to enhance the specificity of the Trk family of receptors for neurotrophins (*2*). $p75^{NGFR}$ has some sequence similarity to the tumor necrosis factor receptors [TNFR I (*3*) and TNFR II (*4*)], the human cell surface antigen Fas (Apo-1) (*5*), and the B cell antigen CD40 (*6*), all of which mediate cell death. In the case of TNFR I and Fas, binding of the receptor by ligand or antibody initiates cell death. In the case of CD40, however, binding by monoclonal antibody (mAb) or ligand inhibits cell death (*6*). Thus, some cells expressing CD40 are dependent on ligand or mAb binding for survival. Because of structural and functional analogies between the CD40 and $p75^{NGFR}$ systems, the possibility that $p75^{NGFR}$ serves as a constitutive cell death–promoting molecule that is inhibited by NGF binding was evaluated.

We expressed $p75^{NGFR}$ in temperature-

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Fig. 1. Expression of rat $p75^{NGFR}$ by transfected conditionally immortalized neural cells (*28*). (**A**) Northern blot demonstrating lack of Trk A and $p75^{NGFR}$ expression by CSM 14.1 cells. $p75^{NGFR}$ expression was demonstrated after transfection by pBabe-puro-$p75^{NGFR}$. Lane 1, CSM 14.1 transfected with pBabe-puro-$p75^{NGFR}$ and grown in serum-containing medium without NGF; lane 2, CSM 14.1 transfected with pBabe-puro-$p75^{NGFR}$ and grown in medium with serum and NGF (2 nM); lane 3, CSM 14.1 transfected with pBabe-puro and grown in serum-containing medium without NGF; lane 4, CSM 14.1 transfected with pBabe-puro and grown in medium with serum and NGF (2 nM); and lane 5, PC12 control. Note that the endogenous transcript in PC12 cells [3.7 kb (*29*)] is shorter than the transcript in the pBabe-puro-$p75^{NGFR}$–transfected cells (predicted to be 4.3 kb), and that treatment of the CSM 14.1 cells with NGF did not result in $p75^{NGFR}$ expression (lanes 2 and 4). Lanes 1 through 4 contained 25 μg of total RNA; lane 5 contained 10 μg of total RNA. (**B**) Immunocytochemistry of CSM 14.1 cells transfected with pBabe-puro-$p75^{NGFR}$ (85 ± 11% of cells expressed $p75^{NGFR}$). (**C**) Immunocytochemistry of CSM 14.1 cells transfected with pBabe-puro (0.6 ± 0.5% of the cells expressed $p75^{NGFR}$). Magnification, ×400.

sensitive immortalized neural cells (*7*) by means of a retroviral vector, pBabe-puro-$p75^{NGFR}$ (*8*) (Fig. 1). Control cells transfected with pBabe-puro expressed neither $p75^{NGFR}$ nor Trk A (Fig. 1). In cells cultured in medium containing serum, expression of $p75^{NGFR}$ had no effect on cell death, but when serum was withdrawn to induce apoptosis (*9*), expression of $p75^{NGFR}$ led to an increase in neural cell death (Fig. 2). However, if NGF (5 nM) was added, not only was the negative effect on cell survival suppressed, but the cells had a death rate less than that of control cells transfected with the identical vector lacking the $p75^{NGFR}$ sequence (Fig. 2). Binding of $p75^{NGFR}$ by a mAb also suppressed the enhancement of neural cell death by $p75^{NGFR}$, but led to less improvement of cell survival than did NGF (Fig. 2). Addition of a control mAb did not affect cell survival (Fig. 2). Neither NGF nor mAb affected survival of the control cells (Fig. 2).

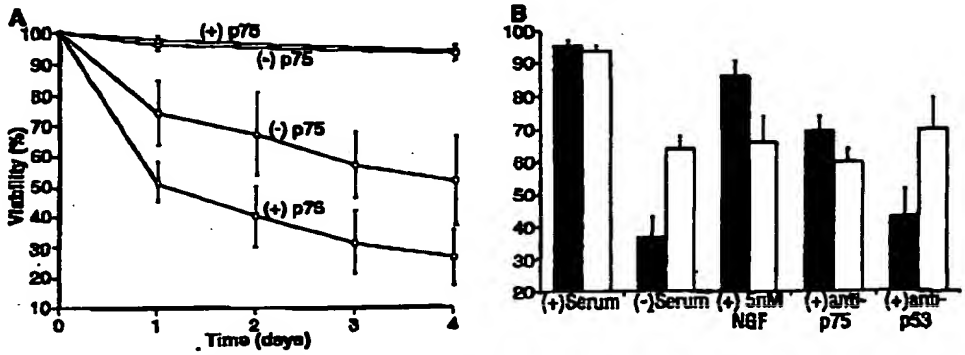
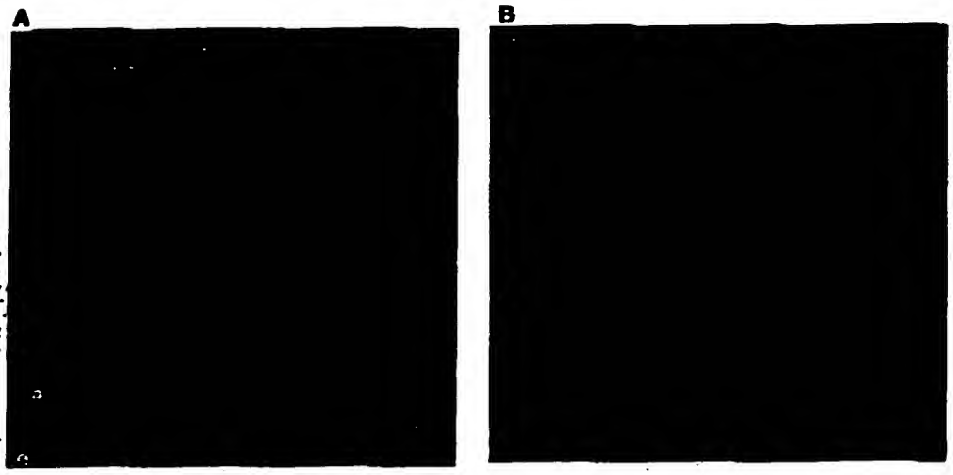


Fig. 2. Enhancement of neural cell death in cells expressing $p75^{NGFR}$. CSM 14.1 cells (*7*) were grown in DMEM with FBS (10%) at 34°C and then switched to the restrictive temperature of 39°C for 36 hours. Cell death was then induced by replacement of the medium with serum-free DMEM [either alone or in combination with NGF (5 nM)], and cell viability was determined each day for 4 days. Viable cells were identified by trypan blue exclusion and by propidium iodide fluorescence. Differences between cells expressing $p75^{NGFR}$ and control cells were highly statistically significant ($P = 0.0001$ by two-way analysis of variance, $n = 5$, from four different stable transfections of each plasmid). Error bars represent standard deviations. (**A**) Effect of serum-free medium on viability of cells expressing $p75^{NGFR}$ in comparison to control cells. Squares, cell transfected with pBabe-puro-$p75^{NGFR}$; circles, cells transfected with pBabe-puro; triangles, cells transfected with pBabe-puro-$p75^{NGFR}$, grown in medium with 10% serum; diamonds, cells transfected with pBabe-puro, grown in medium with 10% serum. (**B**) Effect of NGF (5 nM) and monoclonal antibodies (10 μg/ml) on cells expressing $p75^{NGFR}$ (closed bars) and control cells (open bars). Control mAb was directed against human p53 (anti-p53) (10 μg/ml) (Pharmingen). Each pair showed a highly significant difference ($P < 0.01$ by paired t test, $n = 3$), except the mAb to $p75^{NGFR}$ (anti-p75) ($P < 0.05$) and the controls (no significant difference).

We demonstrated that the type of cell death induced by $p75^{NGFR}$ was apoptotic by expressing $p75^{NGFR}$ in the R2 cell line, a conditionally immortalized cerebellar neural line (*10*) that, in the absence of $p75^{NGFR}$ expression, does not undergo apoptosis in serum-free medium. As shown in Fig. 3, expression of $p75^{NGFR}$ by the R2 cell line led to virtually complete cell death in serum-free medium, with the nuclear fragmentation, chromatin condensation, and homogeneous nuclear staining that are characteristic of apoptosis but not necrosis (*11*). Control R2 transfectants survived well in serum-free medium (Fig. 3).

It was possible that the mediation of neural cell death by $p75^{NGFR}$ might have been a result of the vector-driven expression of $p75^{NGFR}$ in neural cells that do not express endogenous $p75^{NGFR}$. Therefore, PC12 pheochromocytoma cells, which express $p75^{NGFR}$ (Fig. 1) and undergo apoptotic cell death after serum withdrawal (*12*), were studied. In the presence of mAb binding to $p75^{NGFR}$ (10 μg/ml), the number of cells undergoing cell death after serum withdrawal for 3 days was decreased from 78 ± 8% to 13 ± 4% ($P < 0.01$ by paired t test, $n = 3$), whereas the same concentration of control mAb did not affect cell survival. Furthermore, mutant PC12 cells lacking expression of $p75^{NGFR}$ (*13*) underwent very little cell death in serum-free medium (12 ± 6% cell death after 3 days of serum-free medium, $n = 4$), whereas mutant PC12 cells derived in parallel (*13*) that retained expression of $p75^{NGFR}$ also retained the characteristic of undergoing cell death in response to serum withdrawal (50 ± 15% cell death after 3 days of serum-free medium, $n = 4$; $P < 0.01$ by paired t test). As an additional control, another plasma membrane protein, β-amyloid precursor protein (β-APP_{751}), was expressed with the pBabe-puro expression vector in the same conditionally immortalized neural cell line (CSM 14.1), without effect on apoptosis (*14*). This does not exclude the possibility that the expression of other proteins may enhance apoptosis.

Although both NGF and mAb directed against $p75^{NGFR}$ enhanced cell survival, and although Trk A is not expressed by CSM 14.1 cells (Fig. 1A), it was possible that NGF inhibited the death of tempera-



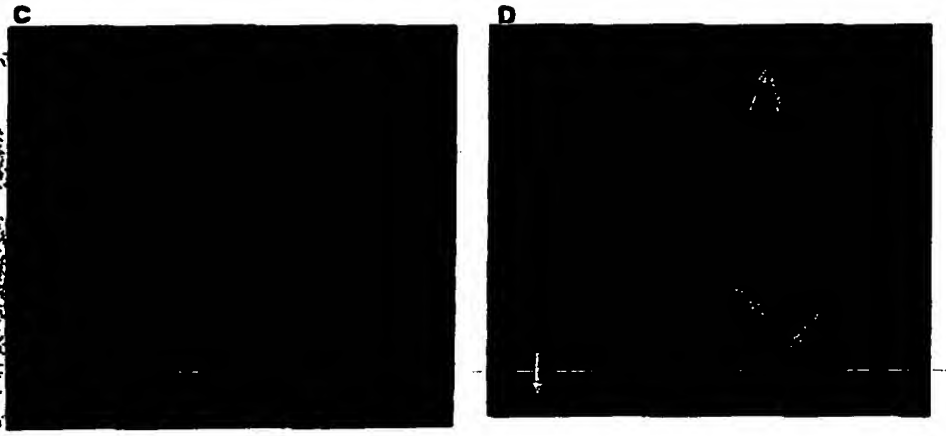


Fig. 3. Apoptosis in R2 cells (*10*) transfected with pBabe-puro-p75^{NGFR}, but not in R2 cells transfected with pBabe-puro. Cells were grown in DMEM with FBS (10%) at 34°C, seeded at a density of 5 × 10^4 cells/cm^2, and placed at 39°C in serum-free medium. After 6 days in serum-free medium, propidium iodide was added at a concentration of 10 μM, and cells were viewed with a Zeiss Axiovert microscope. (A and B) R2 cells transfected with pBabe-puro. (C and D) R2 cells transfected with pBabe-puro-p75^{NGFR}. (A and C) Phase contrast. (B and D) Fluorescence. In (D), many nuclei are fragmented, which is characteristic of apoptosis (single arrows mark some examples); other nuclei are homogeneously stained, also characteristic of apoptosis. The only example of a nonapoptotic nucleus in this field is denoted by a double arrow. Magnification, ×320.

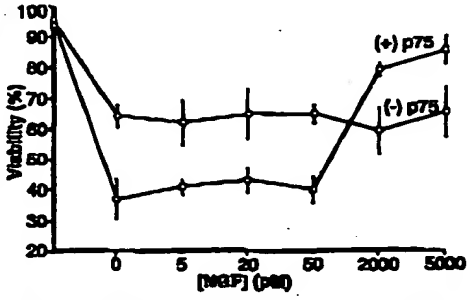


Fig. 4. Inhibition of conditionally immortalized neural cell death by various concentrations of NGF. CSM 14.1 cells were grown as described in Fig. 2. Serum-free medium included the indicated concentrations of NGF. Error bars represent standard deviations (n = 3).

ture-sensitive immortalized neural cells expressing p75^{NGFR} by binding to the high-affinity receptor [dissociation constant (K_d) = 2.3 × 10^{-11} M (*15*)] rather than the low-affinity receptor [K_d = 1.7 × 10^{-9} M (*15*)]. Therefore, several concentrations of NGF were tested. The inhibition of cell death by NGF in this cell line was minimal at concentrations of NGF that bind only the high-affinity NGF receptor significantly (Fig. 4). In contrast, concentrations of NGF equaling or exceeding the affinity constant for binding to the low-affinity receptor increased cell survival (Fig. 4). Survival of control CSM 14.1 cells transfected with the expression construct lacking the p75^{NGFR} open reading frame was not increased by NGF (Fig. 4).

Thus, the expression of p75^{NGFR} resulted in an enhancement of neural cell death in serum-free medium when p75^{NGFR} was not bound by ligand or antibody, whereas enhancement of survival beyond that of controls occurred with binding of the receptor. This dichotomous response defines a previously undescribed type of receptor function within the nervous system. This effect of p75^{NGFR} may account for the fact that some cells become dependent for their survival on the binding of NGF. Early neural cell precursors are independent of NGF, but during development specific neural cells become dependent on NGF (*16*). Increased expression of p75^{NGFR}, which has been shown to occur during development (*17*), could conceivably effect such a switch. Although binding of NGF to Trk A enhances cellular survival and differentiation (*1*), active induction of cell death in the absence of NGF may also occur, and this may be mediated at least in part by p75^{NGFR}. The type of cell death induced by p75^{NGFR}—apoptosis—is the same as that induced by growth factor withdrawal (*18*). However, we cannot exclude the possibility that p75^{NGFR} may under some conditions induce necrosis, especially because the TNFRs may mediate either apoptosis or necrosis (*19*). Our results suggest an additional function for p75^{NGFR} in neural cells, but have no bearing on the other functions ascribed to p75^{NGFR} or on the interaction of other neurotrophins, such as brain-derived neurotrophic factor, with p75^{NGFR}. However, the enhancement of neural cell survival by binding of NGF or mAb to p75^{NGFR} suggests that a similar effect might occur when p75^{NGFR} is bound by other neurotrophins. Neither do the results bear on the role of p75^{NGFR} in the death of non-neuronal cells, such as astrocytes or developing renal cells.

Somewhat similar receptors have been described, including the TNFRs, FAS (Apo-1), and CD40. These molecules show general structural similarity to p75^{NGFR}, with similar extracellular cysteine-rich pseudorepeats and a single transmembrane domain (*20*). The structural similarity of p75^{NGFR} to the other members of the superfamily occurs in the extracellular domain (*5*), but the functional similarity may result from the transduction of a signal leading to (or inhibiting) cell death. The function of p75^{NGFR} is analogous to that of CD40 in that expression occurs on developing cells [mainly central cholinergic, sympathetic, and sensory neurons in the case of p75^{NGFR}, centroblasts and centrocytes in the case of CD40 (*6*)], and leads to a requirement for binding if survival is to occur. In both cases, binding of the receptor leads to improved, but incomplete, cell survival (Figs. 2 and 4) (*6*). Other determinants are clearly involved, because binding of antigen by developing B cells also enhances survival (*6*), lack of expression of CD40 ligand does not result in a reduction

in circulating B cells (*21*), and neural cells expressing $p75^{NGFR}$ survive in media containing serum (Fig. 2). The mechanism by which unbound $p75^{NGFR}$ or other members of this receptor superfamily lead to neural cell death is unknown. However, the structural and functional relation between $p75^{NGFR}$ and TNFR I and II suggests that they may have similar mechanisms of action.

The highest level of expression of $p75^{NGFR}$ in the central nervous system occurs in cholinergic neurons of the nucleus basalis of Meynert, the cells most severely affected in Alzheimer's disease. These cells continue to express normal (*22*) or supranormal (*23*) amounts of $p75^{NGFR}$ mRNA and protein during the neuronal degeneration associated with Alzheimer's disease. In contrast, cholinergic cells of the brainstem that resemble those of the nucleus basalis morphologically, but do not express $p75^{NGFR}$ (*24*), do not degenerate in Alzheimer's disease (*25*).

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28. The $p75^{NGFR}$ cDNA in pUC9 was digested with Sal I, filled in with Klenow fragment and deoxynucleotide triphosphates, and then digested with Bgl II. The 1.7-kb fragment containing the entire open reading frame of $p75^{NGFR}$ was then ligated into pUC18 that had been digested with Sma I and Bam HI. The resulting plasmid was digested with Eco 47III and Sal I and ligated into pBabe-puro (*8*) that had been cut with Sna BI and Sal I, to create pBabe-puro-$p75^{NGFR}$. CSM 14.1 cells (*7*) are rat nigral neural cells immortalized with the temperature-sensitive large T antigen of SV40. These cells express tyrosine hydroxylase, neuron-specific enolase, and neurofilament (NF-L). CSM 14.1 cells were transfected with pBabe-puro-$p75^{NGFR}$ with the cationic lipid DOTAP (Boehringer Mannheim, Inc.) and then selected in puromycin (7 μg/ml). The comparison of single colonies can introduce bias into the results (*9*), but this was obviated by comparison of entire pools of stable transfectants (*9*); therefore, pools of stable transfectants (populations including more than 100 separate colonies) with pBabe-puro-$p75^{NGFR}$ were compared with pools of pBabe-puro transfectants. Cells were grown in DMEM with fetal bovine serum (FBS) (10%) at 34°C in 5% CO_2. Total RNA was prepared by the method of Chomczynski (*26*), and electrophoresis was carried out in formaldehyde gels. After Northern transfer to nylon, ^{32}P-labeled probes for $p75^{NGFR}$ (1-kb cDNA fragment, digested with Stu I), TrkA (0.5-kb cDNA fragment, digested with Xho I), and γ-actin were hybridized sequentially. Blots were exposed to film for 24 hours for the $p75^{NGFR}$ and TrkA probes and for 2 hours for the γ-actin probe. For immunocytochemistry, cells were fixed in paraformaldehyde (4%) for 15 min and permeabilized in 0.1% Triton X-100. Immunocytochemistry was done as described (*27*), with a polyclonal antibody (1:2500) to purified $p75^{NGFR}$. As controls, primary antibody was omitted and control transfectants were stained; both of these controls showed a similar lack of staining.
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Redundant Mechanisms of Calcium-Induced Calcium Release Underlying Calcium Waves During Fertilization of Sea Urchin Eggs

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Propagating Ca^{2+} waves are a characteristic feature of Ca^{2+}-linked signal transduction pathways. Intracellular Ca^{2+} waves are formed by regenerative stimulation of Ca^{2+} release from intracellular stores by Ca^{2+} itself. Mechanisms that rely on either inositol trisphosphate or ryanodine receptor channels have been proposed to account for Ca^{2+} waves in various cell types. Both channel types contributed to the Ca^{2+} wave during fertilization of sea urchin eggs. Alternative mechanisms of Ca^{2+} release imply redundancy but may also allow for modulation and diversity in the generation of Ca^{2+} waves.

Transient increases in the concentration of calcium ions ($[Ca^{2+}]_i$) act as cell signals. In general, the signal shows spatial and temporal inhomogeneity and takes the form of waves or oscillations within the cell (*1*). Several mechanisms have been proposed to account for regenerative Ca^{2+} release (*2*). Release of Ca^{2+} from internal stores can be stimulated by an increase in $[Ca^{2+}]_i$; this process is termed Ca^{2+}-induced Ca^{2+} release (CICR) (*3*). This Ca^{2+} release appears to be mediated by Ca^{2+} channels in the endoplasmic reticulum (ER) that are sensitive to cytoplasmic agonists, to $[Ca^{2+}]_i$, and to the amount of Ca^{2+} in the lumen of the ER (*4*). Two closely related Ca^{2+} channels with these properties are the inositol trisphosphate (IP_3) receptor (IP_3R) (*5*) and the

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